

Jack et al.  
U.S.S.N.: 09/738,444  
Filed December 15, 2000  
Page 4

(c) detecting, purifying or selectively mutagenizing the DNA molecule by means of the target single strand region.

36. (New) A method of claim 35, wherein the two sites bordering the target region are both located on a single strand of the double stranded DNA so that the target single stranded region comprises a gap in the double stranded DNA.

#### **REMARKS**

Claims 1-5 have been amended and new claims 35-36 have been added. The claim amendments and new claims are provided in response to a request by the Supervisory Examiner to add uses described in the specification for creating target single strand regions into the body of the claims. Applicants thank the Supervisory Examiner for his guidance. The amendments provided a basis for an additional search for related prior art. However, no additional references were identified.

No Advisory Action: It is noted that no Examiner's response or Advisory Action concerning Applicant's response to the Final Office Action dated February 11, 2003 was received by Applicants. Hence, Applicants are uncertain that the amendments submitted in that response have been entered. However, the marked up claims submitted herein assume that the claim amendments submitted in the response to the final office action dated February 11, 2003 were indeed entered.

Jack et al.  
U.S.S.N.: 09/738,444  
Filed December 15, 2000  
Page 5

### **CONCLUSION**

For the reasons set forth above, Applicants respectfully submit all rejections have been overcome and that this case is in condition for immediate allowance. Early and favorable consideration leading to prompt issuance of this Application is earnestly solicited. No additional fees are believed to be outstanding as the present amendment is presented during the period of time permitted under the Notice of Appeal. In the event that dues are owed, please debit our Deposit Account No. 14-0740.

Respectfully submitted,

NEW ENGLAND BIOLABS, INC.

Date: 5/9/03

  
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Jack et al.  
U.S.S.N.: 09/738,444  
Filed December 15, 2000  
Page 6

### MARKED-UP VERSION OF THE CLAIMS

1. (amended) A method for creating [a] target single strand regions in a plurality of double stranded DNA molecules for use in joining the DNA molecules, comprising:

(a) nicking at least two sites bordering a target region within the DNA molecules with at least one site-specific nicking endonuclease; [and]

(b) subjecting the DNA molecules from step (a) to conditions [where the target region is] that selectively denature[d] the target single stranded region; and

(c) joining the DNA molecules from step (b) by means of the target single strand regions.

2. (amended) The method of claim 1, wherein the at least two sites bordering the target [single stranded] region are located on a single strand of the double stranded DNA so that the target single strand region comprises a gap in the double stranded DNA. [comprises a gap in the double stranded DNA [ and wherein the method comprises:

(a) nicking at least one site bordering the target region in the double-stranded DNA with at least one site specific nicking endonuclease; and

(b) subjecting the nicked DNA to conditions where the target region is selectively denatured.]

3. (amended) A method for creating a target single strand region at a terminus of a linear double stranded DNA molecule for

Jack et al.  
U.S.S.N.: 09/738,444  
Filed December 15, 2000  
Page 7

use in joining the DNA molecule to a second DNA molecule by means of the single strand region, or for detecting, purifying or selectively mutagenizing the DNA molecule, comprising

(a) nicking at least one site bordering the target region [in] at the terminus of the linear double stranded DNA with at least one site-specific nicking endonuclease; [and]

(b) subjecting the nicked DNA molecules from step (a) to conditions [where] that selectively denature the target region to create the target single stranded region; [is selectively denatured]; and

(c) joining the DNA molecule to a second DNA molecule by means of the single strand region, or detecting, purifying or selectively mutagenizing the DNA molecule by means of the single strand region.

4. The method of claim 3, wherein the [break in the second strand] DNA terminus is pre-existing.

5. The method of claim 3, wherein the DNA terminus [break in the second strand] is formed [produced] by a site-specific endonuclease cleavage.

35. (new) A method for creating a target single strand region in a double stranded DNA molecule for use in detecting, purifying or selectively mutagenizing the DNA molecule, the method comprising:

Jack et al.  
U.S.S.N.: 09/738,444  
Filed December 15, 2000  
Page 8

(a) nicking at least two sites bordering a target region in the DNA molecule with at least one site-specific nicking endonuclease; [and]

(b) subjecting the nicked DNA molecule from step (a) to conditions that selectively denature [where] the target region [is selectively denatured to produce] to create a single stranded region; and

(c) detecting, purifying or selectively mutagenizing the DNA molecule by means of the target single strand region.

36. (new) A method of claim 35 wherein the single stranded region comprises a gap in the double stranded DNA and wherein the two sites bordering the target region are both located on a single strand of the double stranded DNA.